Synopsis

Investigating protrusions in collective cell migration during ovarian development of *Drosophila melanogaster*

by

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Cell migration, single or collective, is essential during development, wound healing, and tissue morphogenesis in multicellular eukaryotes. During any cell migration, the F-Actin structure plays a pivotal role in regulating forward and retraction movements, shape changes, and protrusion formation, which is considerably more complicated due to synchronization and communication required in the case of collective cell migration. Additionally, the regulation of F-Actin in migrating cells is strongly controlled by the successive act of a plethora of 'actin-interacting proteins' like F-actin filament branching factor, the Arp2/3 complex, Nucleation Promoting Factors like WASP, formins and WAVE, GTPases like Rac1, Rho and Cdc42, cross-linkers like alpha-actinin, filamin, fascin, villin, etc., elongating factors like Ena or focal adhesion molecules like Talin and Vinculin. We have used Border Cell Migration in the *Drosophila melanogaster* egg chamber as a genetically tractable model to study collective cell migration. During *Drosophila* oogenesis, a cluster of 6-10 epithelial cells of the egg follicle migrate as a collective cohort between the nurse cells to reach the oocyte nucleus. This cluster of cells forms from the epithelial layer covering the egg chambers and retains many epithelial cell characteristics, albeit at the cluster level and not at the cellular level. During migration, these clusters show distinct characteristics like 'shape' and 'protrusions' that are uncommon within the known collectively migrating cells. Literature studies revealed that cellular shape and extensions, like protrusions, are regulated by F-Actin and actin-interacting proteins. This work tried to understand the roles of some of those actin-interacting proteins during border cell migration. We have used genetic tools to understand the roles of individual genes/proteins as well as interactions among more than one gene/protein. This approach allows the study of the roles of genes *in vivo*, albeit the final assays are *ex vivo*. In this approach, border cells develop with less or no gene product(s) and show the effect as phenotypes. However, this approach does not specify if the gene(s)/protein(s) is/are interacting physically. In this thesis, we concentrated on genetic interaction studies of Actin-interacting proteins. Through this study, we tried to decipher the significant roles of three key Actin-interacting proteins - parallel Actin bundles cross-linker Singed (vertebrate homolog Fascin), filament branching protein Arp2/3 complex and Focal Adhesion Molecule, Vinculin. We found many genes through a targeted screening to find the redundant gene(s) that work with *singed* and decided to study only *vinculin* and the *Arp2*/3 complex genes. We observed that *singed* alone does not affect border cell migration nor *vinculin*. However, the absence of both *singed* and *vinculin* affects border cell migration modestly but statistically significantly. The genes of the *Arp2*/3 complex show moderate effects

on border cell migration; with *singed*, the effect is tremendous. Both *singed* + *vinculin* and *singed* + *Arp2* depleted border cell clusters show significantly decreased F-Actin. Altering the F-actin level has brought about noteworthy changes in the morphological characteristics of border cells. Border cells with less *singed* + *vinculin* show 'shorter' and 'narrower' 'front' protrusions and a more significant number of 'side' protrusions. On the other hand, border cells with less *singed* + *Arp2* do not show significant changes in protrusion characteristics. Instead, *singed* + *Arp2* depleted clusters impact Actin dynamics and diffusive motion in BC clusters, making the cluster 'rounder' and 'wider' with more 'double protrusions' and a lesser diffusion coefficient of F-actin during BCM. We not only focused on these three genes only but found fantastic genetic signaling, for the first time, which regulates BCM. We found that *Rac1* may act upstream of *Rok* while *singed* may act downstream of *Rac1* and *Rok*. We have also found that *singed* possibly works downstream of the Arp2/3 complex while the *Arp2*/3 complex works upstream to *Ena*. However, the data also indicated that *Rok* or *Rac1* cannot regulate Arp2/3 complex genes. We have also found that the NPF *WASp* is possibly controlled by upstream molecules *Rac1* and *Rok* while *WASp*, in turn, may be influencing its downstream collaborator, *singed*. In the long run, we want to conclude that we have tried to present a better version of the Singed protein, showing redundancy and the Arp2/3 complex, and Vinculin is its potential genetic partner. Additionally, we have contemplated that they can genetically team up among themselves and simultaneously with *Rac1*, *Rok*, *WASp* and *Ena* to control border cell migration. This, in turn, affects the cluster's stiffness, viscosity, shape, and area. Furthermore, we also believe that BCM regulation is a vigorous process with built-in redundancies. We have also seen in the literature that Singed, Vinculin, and the Arp2/3 complexes have become potential candidates for use as novel prognostic biomarkers in therapeutic studies. Therefore, our research suggests that our novel approach may decipher a therapeutic tactic that can prevent the metastatic conversion of adenoma to adenocarcinoma. Also, we have seen that double knockdown of both genes has displayed large clusters that are stiff, viscous and could not migrate. Hence, our study can be used to check the metastatic growth of tumours and the signalling pathway, which we have found can be used as a budding therapeutic target in several kinds of cancer.